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Report

Twisting of Neocortical Progenitor Cells Underlies a Spring-like Mechanism for Daughter-Cell Migration

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Summary

The mammalian neocortical wall thickens extensively during embryogenesis via proliferation of progenitor cells [1–4] and migration of daughter cells toward the pial surface [5–8]. Time-lapse imaging and functional experiments were carried out so that the possible involvement of mechanical forces in these processes could be examined. When bipolar cells connecting the ventricular and pial surfaces of the mouse cerebral wall lose their ventricular attachment, they undergo somal translocation toward the outer zones, which contain differentiated neurons. The pial process of these transitioning unipolar cells exhibits a coiled or hairpin-loop morphology, suggesting that twisting and stretching of the pial process establishes a spring-like mechanism that propels the daughter cell toward the pial surface upon ventricular detachment. This model is supported by morphological changes observed in microsurgically transected pial processes. Pharmacological experiments further reveal the involvement of intermediate filaments in twisting of pial processes. These results uncover a novel mechanism for cellular migration and provide valuable tools for the detailed study of the role of mechanical forces in 3D brain development.

Results and Discussion

Morphology of Transitioning Unipolar Cells in the Embryonic Neocortex Suggests a Spring-like Force in the Pial Process

Thickening of the neocortical primordium (Movie S1 in the Supplemental Data available with this article online) is achieved by a number of cellular events. These include proliferation of progenitor cells ("radial glia"), which are bipolar cells whose elongated processes connect the pial and ventricular surfaces [1–4] (Movie S2), as well as migration and maturation of daughter neurons [5–8] (Figure 1A). However, our understanding of dynamic cellular behavior in three-dimensionally crowded tissues is still limited. Furthermore, we do not know how mechanical forces work to shape neocortical architecture. Although physical forces are known to be important for proper formation of the neural tube and brain vesicles from the neuroectodermal sheet [9–11], their involvement in subsequent cellular behaviors during brain wall thickening has not been directly examined.

Previously, we observed in slice cultures that daughter cells of embryonic day 14 (E14) neocortical progenitor cells undergo a novel mode of migration [2, 4]. Progenitor cells divide at the ventricular surface without losing their pial process (Movie S2), which can be inherited by both neurons [2] and lineage-restricted (non-stem-like) progenitor cells [4]. Daughter cells are bipolar shortly after birth but become unipolar upon collapse of the ventricular process and concomitantly migrate toward the differentiating cell zones (Figures 1A–1F; Movie S3 and Figure S1).

In "locomotion" [6–8], a migratory mode used by neurons with a leading process (up to 100 μm long) free of the pial surface (Figure 1A), centrosome-dependent "nuclear pulling" is thought to be important [12, 13]. To determine whether a similar process functions in somal movement during the bipolar-to-unipolar (B-U) transition of neocortical cells, we examined the position of γ-tubulin-positive centrosomes in unipolar cells by immunohistochemistry (Figure 1B and Figure S1F). Strikingly, all cells examined (n = 7) contained a centrosome in the tailing (ventricular) process but not in front of the nucleus as in locomoting neurons, and such findings are consistent with previous electron microscopic observations [14]. This suggests that cells undergoing the B-U transition may have a unique strategy for somal movement.

Interestingly, we observed that the pial process of the B-U transitioning cells frequently appeared to be bent (35%), curled or sinuous (73%), or hairpin-loop shaped (24%) (n = 62; Figures 1C–1F; see also Figure S1 and Movies S3 and S4). These morphologies were highly characteristic of transitioning unipolar cells in slice cultures (Figure S1E), and in freshly fixed in vivo specimens, the pial processes of unipolar cells showed significantly higher "sinuosity" than did pial processes of bipolar cells (Figure S2). Furthermore, during the somal translocation phase, when sinuous or hairpin morphologies were most noticeable, the length of the pial process was almost constant (Figure 1F and Figure S3). In a subsequent phase, the somal migration distance was observed to be almost equivalent to the length of pial-process shortening. Although this latter phase can be explained by a simple insertion of the nucleus into the pial process, as in the nuclear-pulling model for locomoting neurons, the somal translocation phase characterized by both isometry and sinuosity of the pial process requires different, or at least modified, explanations.

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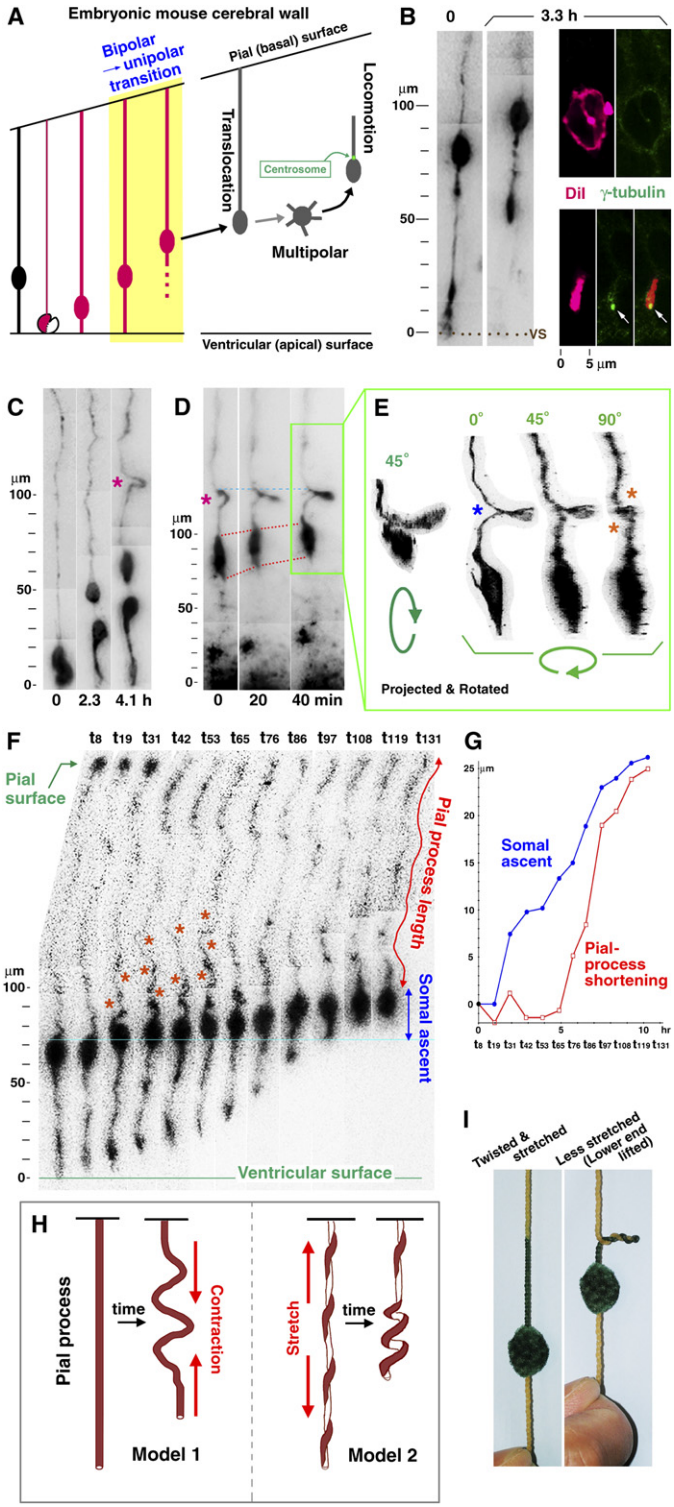

Figure 1. Migration and Pial-Process Morphology of Transitioning Unipolar Cells

(A) Illustration of the midgestation mouse embryonic neocortical wall (150–300 μm thick) and daughter-cell behavior. A daughter cell (red) is initially bipolar in shape, having inherited its pial process from the progenitor cell (black). It then loses its apical attachment, adopting a unipolar morphology, and this bipolar-to-unipolar transition was accompanied by translocation of the soma. This phenomenon (shadowed in yellow) may be followed by translocation, multipolar migration, and locomotion, although the relationships between these are only partially understood. In locomoting neurons, localization of a centrosome in front of the nucleus and a centrosome-dependent "pulling" model has been proposed for explaining nuclear movement.

(B) Anti-γ-tubulin immunostaining (green) of a bipolar-to-unipolar transitioning cell in slice culture reveals that it has a centrosome in its tail.

(C) Hairpin-loop morphology (red asterisk) of the pial process. One daughter cell that inherited the pial process ascended more quickly than its sister cell. Separate confocal microscopic examinations revealed that the upper daughter cell had lost its apical attachment by 4.1 hr (not shown).

(D and E) Another example of hairpin-loop formation (red asterisk) in the pial process. In (D), the distance between the top of the curve (0 min) or loop (40 min) and the soma was almost constant, and the extent of somal movement (11 μm) and the degree of loop growth were correlated, suggesting winching of the soma. Note also that a single pial process can exhibit several different morphologies depending on the angle of observation.

(F) Time-lapse images of a transitioning unipolar cell (see also Movie S3). Numbers at the top represent time points (5 min intervals). Asterisks indicate sinuous or coiled-like morphologies.

(G) Graph representing somal translocation and shortening of the pial process of the cell in (F) (see also Movie S3). During the initial observation period, when somal movement toward the pial side was not accompanied by pial-process shortening, the pial process was the most sinuous.

(H) Two models explaining the morphology and mechanics of the pial process.

(I) Simulation involving a rubber string with a soma-like sponge (green colored for comparison with [D] and [E]).

We suggest two possible mechanisms that might explain the movement of the daughter-cell soma (Figure 1H). In the first model, the pial process contracts, causing release of contact at the ventricular surface, movement of the soma, and buckling of the process, as previously observed in axons of monolayer-cultured neurons [15]. In the second model, the pial processes of bipolar cells are twisted, but this twisting is not readily noticeable because the processes are stretched. Translocation of transitioning unipolar-cell somata is the result of a spring-like mechanism provided by twisting and stretching of the pial process and its subsequent release after the loss of the ventricular process. The latter model is consistent with the helical and coiled-like structures seen in cells in slice cultures (Figure 1 and Movie S4) and macroscopic simulations with rubber

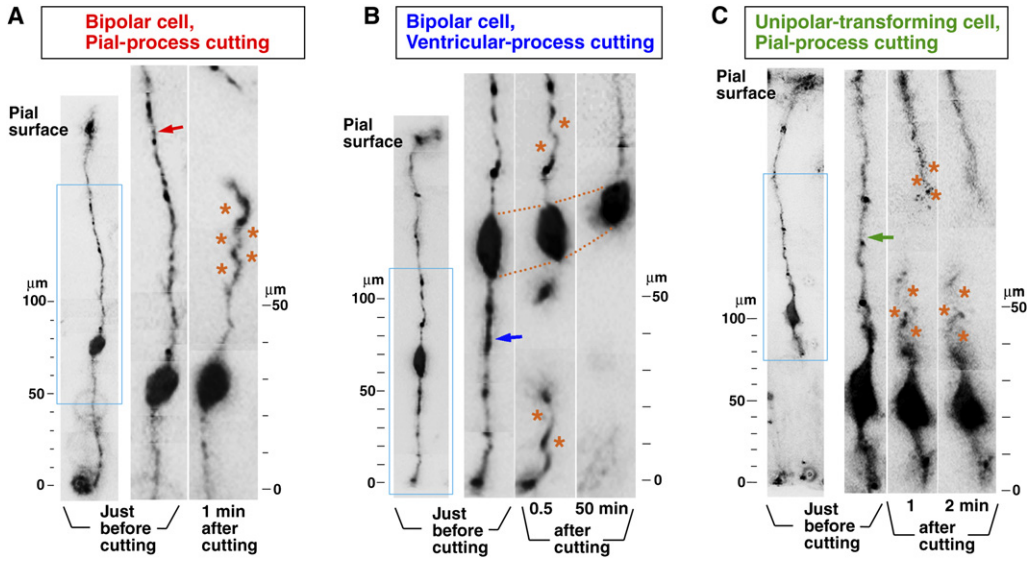

Figure 2. Process Transection Leads to Formation of Process Coils

Pial (A and C) or ventricular (B) processes of bipolar (A and B) or unipolar (C) cells were transected with microcapillaries. The processes were transected at the sites indicated by arrows.

strings (Figure 1I) and silicone tubes (Figure S4A). These two models are not mutually exclusive.

Microsurgical Experiments Strongly Support the "Twist-Plus-Stretch" Model

To determine whether the processes of bipolar cells are twisted and stretched and to test whether a spring-like mechanism functions in cell migration, we designed three types of microsurgical experiments (Figure S4B). First, we severed pial processes (1–2 μm in diameter) by using DiI-labeled glass capillaries (1–2 μm in diameter at the tip). The pial (distal) or proximal cellular material adopted a sinuous or coiled-like appearance within 1–2 min after transection (91%, n = 174) (Figure 2A; see also Figure S5 and Movies S5 and S6), as predicted from simulations with rubber strings (Figure 1I). The distance between the transected ends increased for an additional 5–8 hr (100%, n = 29), but the sinuous appearance became less prominent over time. In 38% (11/29) of cases in which transected cells were monitored over a longer period of time, somal translocation toward the ventricular surface was observed (Figure S6A). Phase-contrast microscopy at 1–2 min revealed no tissue gaps between the proximal and distal transected ends (Figure S7). We confirmed that the transected processes exhibited a sinuous morphology simply because of intrinsic physical properties and not because of lateral compression from the surrounding tissue (Figure S7).

Second, we transected the ventricular process of bipolar cells (Figure 2B, n = 33). If bipolar cells undergo the proposed twist-and-stretch mechanism, transection of the ventricular process should cause the remaining pial process to adopt a sinuous appearance. Furthermore, some abventricular somal translocation should be seen if the spring-like mechanism functions during normal migration of the B-U transforming cells. In the vast majority of cells examined, the pial process adopted a sinuous appearance (88%), and slight somal translocation was noted (79%) within 1–2 min after process transection. In 53% of cases in which cells were monitored over a longer period of time (n = 17), further translocation occurred predominantly within 1 hr of transection, with similar kinetics to those of normal B-U transitioning cells (Figure S6B).

Third, we transected the pial process of unipolar cells (n = 40). Our model predicts that if hairpin-loop formation in the pial process of transitioning unipolar cells is the *cause* but not the *result* of somal translocation, coil formation should be visible after transection of the pial process of unipolar cells (Figure 2C). This was indeed the case (65%).

To confirm that processes were twisted, we examined them by high-resolution confocal microscopy and scanning electron microscopy (SEM). Three-dimensionally projected images obtained through confocal microscopy with a step size of 0.1 μm showed parallel lines running oblique to the axis of live DiI-labeled processes (Figure 3A and Figures S5B and S8A), and such a morphology was best explained by twisting of the processes. Processes with a very similar morphology were detected by SEM (Figures 3B and 3B′) and are readily recognizable in previously published SEM photomicrographs [16]. Similar parallel lines were also observed in less magnified images of pial processes of B-U transitioning cells in vivo (Figure 3C) and in the tip of newly growing pial processes both in vivo (Figure 3E) and in slices (Figure 3F and Figure S8B).

Together with our observations of the morphological and migratory characteristics of cells in the neocortex, these microsurgical experiments indicate that twisting and stretching of the pial process contribute to soma movement during the B-U transition by providing a spring-like force (Model 2 of Figure 1H). This spring-based mechanism may be most important during the initial step of daughter-cell migration, when cells move

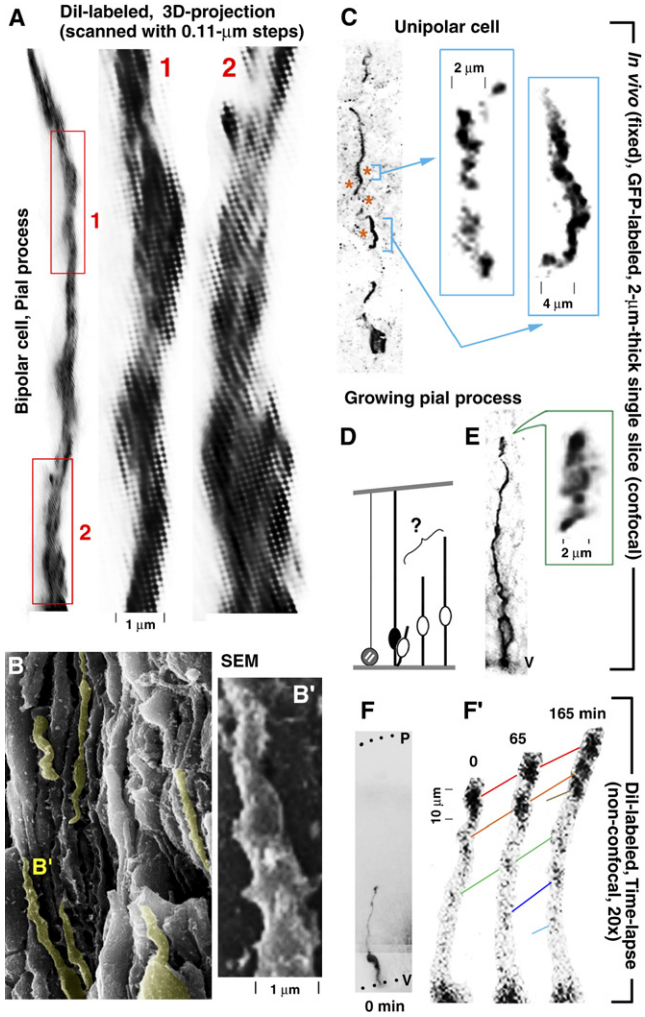

Figure 3. Pial Processes Are Twisted

(A) Three-dimensionally projected image of a normal (uncut) pial process labeled with DiI and captured by high-resolution confocal microscopy.

(B and B′) Scanning electron micrograph showing sinuous and twisted-like processes (pseudocolored yellow) in a cerebral wall.

(C) A unipolar cell in an E14 cerebral wall that was electroporated with a GFP-encoding cDNA 1 day earlier. Magnified views of the sinuous regions (asterisks) show a twisted-like morphology characterized by parallel lines.

(D) Illustration showing a progenitor cell extending a process (white soma). Morphology of the growing process (question mark) was examined both in vivo (see [E]) and in vitro (see [F]).

(E) Growing pial process in vivo. A progenitor cell in an E14 cerebral wall that was electroporated with a GFP-encoding cDNA 1 day earlier appears to exhibit right-handed twisting at the distal end.

(F and F′) Live observation (5 min intervals) of a growing pial process (DiI-labeled). Colored lines identify the behaviors of intensely labeled regions of the process. These regions increased in number and migrated upward with time, with behavior reminiscent of the thread of a left-handed screw (see Figure S6B for images with better time resolution).

away from the ventricular zone, and not during the entire course of daughter-cell migration. During this initial phase, cells with a pial process generally migrate faster than those without a pial process (Figure S6B) [17]. We observed some rotation in the upward-moving somata in our macroscopic simulations (Figure 1I), but we were not able to determine whether such rotation occurred in transitioning unipolar cells by live imaging performed under normal conditions or in the transection experiments. Further detailed studies are needed to clarify this issue.

Intermediate Filaments and Not Microtubules or Microfilaments Are Important for Pial-Process Twisting

To examine the contribution of cytoskeletal elements to the twisting of pial processes, we performed a series of pharmacological experiments combined with microsurgery (Figure 4). In slices treated with nocodazole (10 μg/ml), a drug that causes thinning of the neuroepithelium [18], bipolar cells were dramatically shortened, with frizzy or coiled-appearing pial processes (98%, n = 50; Figure 4A); these data suggest that microtubules are involved in the extension of the pial and ventricular processes but not process twisting. Treatment of slices with cytochalasin D [19] (Figure 4B, 10 μg/ml) or blebbistatin [20] (Figure 4C, 100 μM) for up to 1.5 hr did not cause abnormal pial-process morphology. After transection, processes adopted a sinuous appearance with similar kinetics and frequency as observed in untreated slices (89%, n = 44 for cytochalasin D; 81%, n = 32 for blebbistatin). These data suggest that microfilaments and myosin II are not strong contributors to pial-process twisting.

Vimentin and Nestin are intermediate filament (IF) proteins that are enriched in bipolar progenitor cells [21] and interact with one another [22, 23]. Therefore, we next used calyculin-A, a phosphatase inhibitor known to disassemble IFs in monolayer-cultured cell lines [24], to examine whether IFs are involved in bipolar-cell twisting. All calyculin-treated bipolar cells (n = 61) shortened (Figure 4D), and although the ventricular process was always intact, the pial process frequently (74%) retracted from the pial surface without exhibiting any sinuous appearance (Figure S9). Severing these shortened calyculin-treated pial processes resulted in slight curling (61%, n = 44), but the magnitude of curling was considerably less than was observed in cytochalasin D- or blebbistatin-treated processes.

For a more objective and quantitative assessment, the degree of transection-induced enhancement of

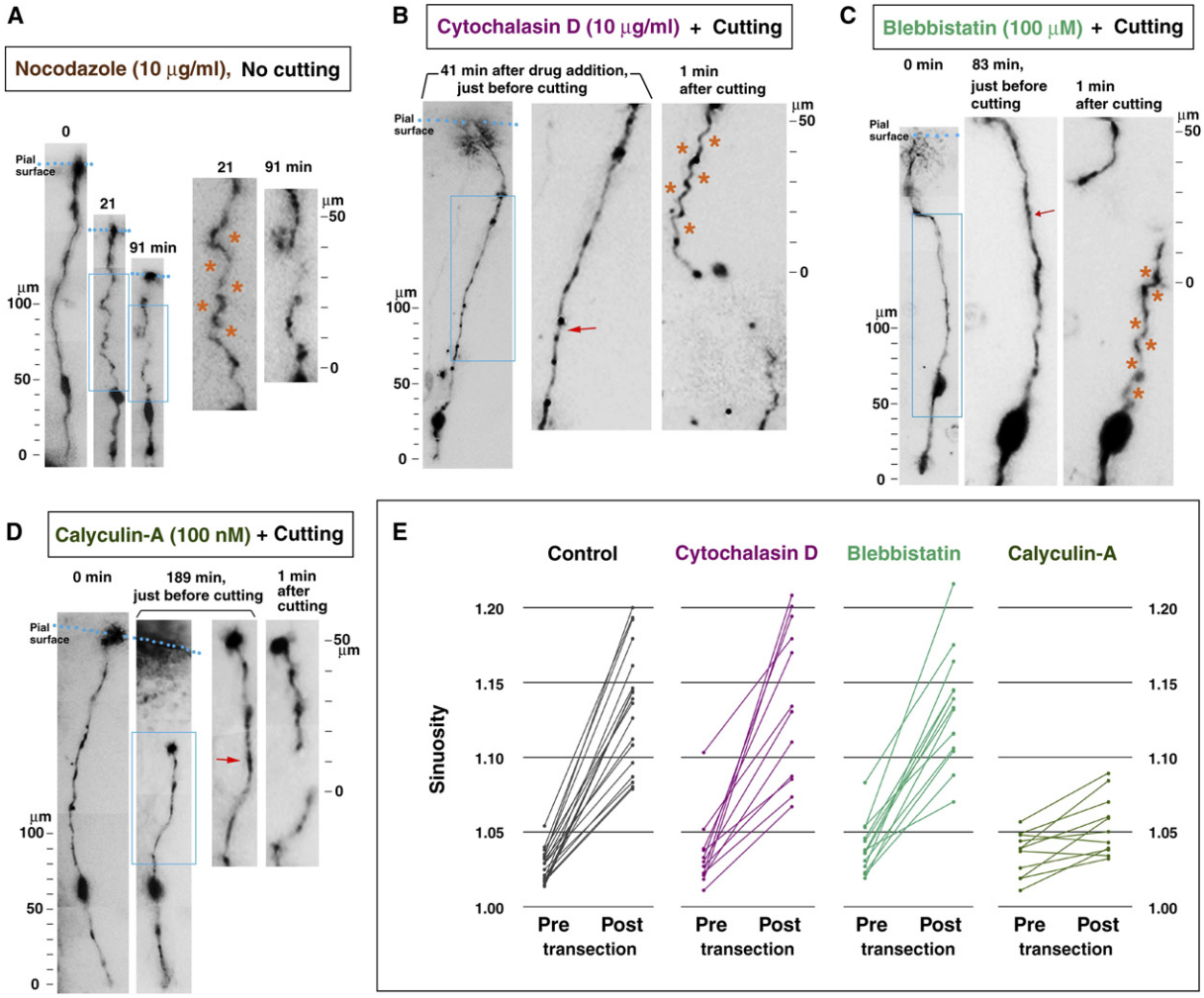

Figure 4. Intermediate Filaments Promote Bipolar-Cell Process Twisting

(A) Morphological change of a bipolar cell treated with nocodazole.

(B–D) Bipolar cells were transected in the presence of cytochalasin D (B), blebbistatin (C), or calyculin-A as an inhibitor of intermediate filaments (D). Arrows indicate the transected portion.

(E) shows that the "sinuosity enhancement index" (SEI) (posttransection sinuosity/pretransection sinuosity) was significantly smaller ($p < 0.0001$, Mann-Whitney's U test) in cells transected after treatment with calyculin-A (1.020 ± 0.016, mean ± SD, n = 11) than in cells of any other group (1.098 ± 0.032 in the control, n = 20; 1.084 ± 0.037 in cytochalasin D, n = 12; 1.092 ± 0.028 in blebbistatin, n = 13). The SEI was obtained for cells randomly chosen from each experimental group with the method shown in Figure S2.

sinuosity in the pial process was compared between the control, cytochalasin D-treated, blebbistatin-treated, and calyculin-A-treated groups (Figure 4E). The measured "sinuosity enhancement index" (posttransection sinuosity/pretransection sinuosity) was significantly smaller in cells transected after treatment with calyculin-A than in cells of any other groups. These results suggest that IFs may participate in both the establishment of bipolar morphology and process twisting.

IFs generally have long half-lives roughly equivalent to the cell-cycle length [25, 26] and are thought to function as mechanical shock absorbers [27]. IF-based twisting might provide rigidity to the cell process, and perhaps function as a cytoskeletal reinforcement factor, and it could be effective to the possible contraction-based mode of somal translocation (Model 1 of Figure 1H). Our preliminary experiments showed that treatment of slices with actomyosin inhibitors for 5–10 hr affected the B-U transition somewhat (data not shown). However, because the culture time for observing B-U transitioning cells is much longer than what is required for transection experiments, longer exposure to actomyosin inhibitors inevitably caused tissue deformities. Thus, we could not determine whether the observed effect on the B-U transition was a consequence of altered cell migration or tissue deformation.

Thus, our data suggest that IF-mediated twisting of pial processes in nascent neocortical cells produces a spring-like force that functions to propel the cell soma away from the proliferating ventricular zone. It is possible that the centrosome-dependent pulling mechanism that plays a role in nucleokinesis in locomoting neurons [12, 13] functions during a later stage of unipolar cell migration, after complete retraction of a tailing process. However, more work must be done to investigate this possibility.

Conclusion

By using mouse neocortical primordia as a tool to examine how mechanical forces shape the brain, we found that bipolar cells spanning the cerebral wall are twisted and stretched, developing a spring-like force. Stretching of these twisted processes appears to be mediated not only actively by the intrinsic cytoskeletal elements, whose production may be regulated in a cell-cycle-dependent and differentiation-dependent manner, but also passively by external forces that reflect the degree of cell crowding in the thickening neocortical wall. The ability of tissue tension [28, 29] or other physical mechanisms [30] arising in a mass of cells to control the integration of individual cells into a developing tissue suggests that mechanical forces play an important role in the development of properly ordered tissues, including the neocortex.

Supplemental Data

Supplemental Data include Experimental Procedures, nine figures, and six movies and can be found with this article online at http://www.current-biology.com/cgi/content/full/17/2/146/DC1/.

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